2546-Pos Board B238
Measurement of Transmembrane Voltage using the Fluorescent Sensitive Dye Annine-6
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Intense electric pulses are known to induce several effects on biological cells and membranes. Among the different effects, membrane electropermeabilisation has been the most studied during the past decades. Until today, electropermeabilisation was reported for all tested pulse duration: from nanoseconds to milliseconds.
It is well accepted that electropermeabilisation is triggered by a modulation of the transmembrane voltage which is itself induced by the electric field. The high induced transmembrane voltage results in high electric field in the membrane. Under such a physical stress, cell membrane becomes permeable reversibly or irreversibly, depending on the strength of the stress. We propose in this study to measure the transmembrane voltage induced by an external electric field. The measurement of the transmembrane voltage is based on the fluorescence emitted by a voltage sensitive dye, ANNINE-6, which incorporates in cells membrane. We specifically address in this work the impact of one single pulse of 100 µs on the mammalian cell line DC3F. We discuss in this study the importance of the calibration of the fluorescence response.

Membrane Fusion I

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Experimental Investigations of Single Liposome to Supported Bilayer Binding Events
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One of the primary ways in which cells interact with their environments is by release of extracellular vesicles. These cell-derived submicron sized containers are formed either from the cell plasma membrane (microvesicles) or secreted from multivesicular bodies (exosomes). These vesicles contain nucleic acids and proteins that have been suggested to play an important role in intercellular signaling and the process of molecular communication between cells. In microvesicle-mediated intercellular communication, vesicles released by a donor cell must bind to the plasma membrane of a recipient cell in order to deliver their cargo to the target. Despite the important physiological role of vesicle-plasma membrane fusion and vesicle endocytosis, the details of the physical interactions between microvesicles and the plasma membrane are still poorly understood. To better understand the forces which occur between microvesicles and cells, we experimentally investigate single liposome binding events with a supported lipid bilayer. We employed total internal reflection fluorescence (TIRF) microscopy to observe liposome interactions with a supported lipid bilayer, and compare the experimentally observed bond lifetimes to the theoretical estimates. Our experimental observations indicate that bond lifetimes increase with both increasing liposome size and decreasing temperature.

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Peptide Nano-Capsules with Lipid Vesicle like Characteristics
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In a recent publication (Gudlur et al., (2012) PLOS ONE, 7 (9) e45374), we described a nano-capsule made from the assembly of a mixture of two poly-cationic branched amphiphilic peptides bis(FLIVI)-K-K_4 and bis(FLIVIGSII)-K-K_4 that have a molecular architecture analogous to that of phospholipids. These peptides self-assemble in water to form hollow structures capable of trapping solutes. The ability of these peptides to assemble into bilayer-delimited spheres is a function of the unique nature of these peptide sequences - that reversibly transition from an alpha helical conformation in 2,2,2-Trifluoroethanol, to a beta sheet in water. These flexible capsules possess many of the properties of phospholipid vesicles such as fusion, solute encapsulation and an ability to be resized by membrane extrusion through polycarbonate filters with defined pore sizes. Here, we demonstrate the biophysical characteristics of these nano-capsules; including, their mode of assembly, the properties of the bilayer defined by the branched hydrophobic sequences, high thermodynamic stability, kinetics of fusion, and their ability to retain their cargo in cellular systems for an extended duration of time without any apparent degradation. The capsules can - like their lipid counterparts - not only be resized, but also maintained there at by reducing the temperature to 4° C, owing to what appears to be a temperature dependent conformational transformation. Moreover, these biomaterial constructs seem to retain their structural integrity even when subjected to alpha particle emissions. The versatility of these peptide nano-capsules lies in our ability to modify the individual peptide sequences with ligands and molecular markers; making these constructs potentially desirable as biocompatible vehicles for the targeted delivery of cargo into the cells.
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Lipid Splay in the Presence of Fusogenic Peptides
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Membrane fusion or fission are ubiquitous processes that allow cells to incorporate material from extracellular space, secrete matter into the environment or even merge.
Models of fusion suggest that the rate limiting step in the fusion process is the bending of acyl chains into the water barrier separating the apposed membranes. This phenomenon is called lipid splay and is thought to precede fusion stalk formation.
Although lipid splay has been observed in several molecular dynamics simulations it has not previously been detected experimentally.
We investigate the splay ionduced by LV peptides. These peptides have previously been characterized in terms of their differential backbone dynamics and fusogenicity in our laboratory [1]. The peptides LV16 and L16 were reconstituted in small unilamellar vesicles comprising three different phospholipids. We established a fluorescence dequenching method that allows us to discriminate between splay efficiencies of these phospholipids in the model liposomes. The results of the splay assay showed that phosphatidyl choline splay exceeds splay of phosphatidyl ethanolamine or phosphatidyl serine. These results were observed for both the LV16 and the L16 peptide.
Furthermore, peptide-lipid interaction was assessed using brominated lipid as a quencher for tryptophan fluorescence of the peptides. The results of our peptide-lipid interaction assays showed that phosphatidylcholine binds more strongly to both the LV16 and the L16 peptides compared to phsophatidyl ethanolamine or phosphatidyl serine.
Due to our peptide-lipid interaction and splay assay results, we assume that phosphatidylcholine plays a key role in the fusion process.
Literature
Reference
[1] Quint et al., Residue-Specific Side-Chain Packing Determines the Backbone Dynamics of Transmembrane Model Helices, Biophysical Journal, 99, 2010.

2550-Pos Board B242
Alternate Splicing of Dysferlin C2A Confers Ca^{2+}-Dependent and Ca^{2+}-Independent Binding for Membrane Repair
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Dysferlin plays a critical role in the Ca^{2+}-dependent repair of micro-lesions that occur in the muscle sarcolemma. Of the seven C2 domains in dysferlin, only C2A is reported to bind both Ca^{2+} and phospholipid thus, acting as a key sensor in membrane repair. Dysferlin C2A exists as two isoforms, the "canonical" C2A, and C2A variant 1 (C2Av1). Interestingly, these isoforms have markedly different responses to Ca^{2+} and phospholipid. Structural and thermodynamic analyses are consistent with the canonical C2A domain as a Ca^{2+}-dependent, phospholipid-binding domain, whereas C2Av1 would likely be Ca^{2+}-independent under physiological conditions. Additionally, both isoforms display remarkably low free energies of stability, indicative of a highly flexible structure. The inverted ligand preference and flexibility for both C2A isoforms suggest the capability for both constitutive and Ca^{2+}-regulated effector interactions, an activity that would be essential in its role as a mediator of membrane repair.

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Inositol Pyrophosphates Inhibit Synaptotagmin-Dependent Exocytosis
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